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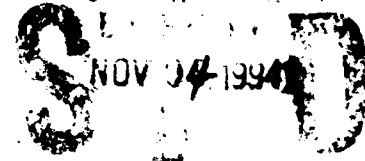
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Increased Susceptibility to Staphylococcal Enterotoxin B Intoxication in Mice Primed with Actinomycin D

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Mice (BALB/cJ, C3H/HeN, and C3H/HeJ) primed with actinomycin D became highly susceptible to lethal intoxication with staphylococcal enterotoxin B (SEB). The mice underwent toxicosis and toxic shock and died. Actinomycin D-primed C3H/HeN and C3H/HeJ mice showed equal sensitivity to SEB, suggesting that bacterial lipopolysaccharide derived from gram-negative bacteria in the gut may not be an important cofactor in intoxication. In a time course study of the illness, prominent pathological changes characterized by blood congestion and thickening of alveolar septa were seen in the lung, while blood congestion, inflammation, epithelial cell flattening, and villous blunting were seen in the small intestine. In lymphoid tissues, such as the spleen, congestion, inflammation, and lymphoid cell depletion were the major reactions. The pathological features of the mice had many similarities to those of rhesus monkeys intoxicated with intravenous SEB. The actinomycin D-primed C3H/HeJ mice are thus an ideal mouse model for studying SEB toxicosis and toxic shock.

Staphylococcal enterotoxin B (SEB) is a superantigen (8, 14). It causes food poisoning when it is ingested and toxic shock and death when it enters the blood circulation and systemic tissues (3, 7, 21, 28). SEB-induced lethal toxic shock appears to be due to functional failures and pathological changes in several organs and organ systems (3, 7, 28). Although cytokines have been suggested to be the causative factors of the toxicosis and toxic shock, the mechanism of pathogenesis remains obscure (4, 9, 10, 11).

Monkeys and chimpanzees are the animal species most sensitive, next to humans, to SEB-induced food poisoning and toxic shock (2, 3). The clinical symptoms and pathological reactions in the monkey and human toxicosis and toxic shock cases are similar (2, 3, 18). We have used monkeys as a toxic shock model to investigate pathogenesis and to test vaccines (25). However, monkeys are expensive, genetically diverse, and limited in numbers and technical approaches. Recently, mice have been used to develop models of lethal intoxication by taking advantages of their relatively low cost and the availability of inbred strains and monoclonal antibody reagents to various cytokines and lymphocyte and leukocyte markers. However, for an unknown reason, mice are resistant to SEB-induced lethal toxic shock. They have to be manipulated if they are to be made susceptible to SEB-induced lethal intoxication. Four methods of increasing SEB sensitivity have been reported: (i) priming with a large dose of D-galactosamine (15); (ii) further intoxication with a large dose of bacterial lipopolysaccharide (LPS) (23, 24); (iii) use of transgenic mice with certain T-cell receptor V β chains (16); and (iv) use of severe combined immunodeficiency mice with transplants of human fetal liver and thymus cells (1). Although all these mice have been proposed as mouse models of SEB intoxication, detailed

pathological examinations of these mice have not been reported. Therefore, the extent of the similarity between the illness in these mice and the illness in monkeys and humans is still unknown.

For an ideal mouse model of SEB toxicosis and toxic shock, pathological reactions similar to those of monkeys or humans are a prerequisite. Actinomycin D (ACT-D) has been used to enhance the sensitivity of L929 cells to tumor necrosis factor in *in vitro* assays (26). It also has an additive effect with LPS and tumor necrosis factor in inducing lethal toxic shock in mice (6). Tumor necrosis factor is thought to be the initiator of the cascade of reactions that leads to toxic shock and death in mice given LPS (5). Because there are similarities in the clinical symptoms of LPS- and SEB-induced toxic shock (3, 24), we have combined ACT-D with SEB and administered the combination to mice in the hope of developing a SEB toxic shock model. The results show that ACT-D indeed enhances SEB in inducing toxic shock and death in mice. The ACT-D-primed mice intoxicated with SEB have many pathological features and clinical signs similar to those of rhesus monkeys challenged intravenously with SEB. The ACT-D-primed mice appear to be a good mouse model of SEB-induced toxic shock.

SEB prepared by the method of Schantz et al. was the same preparation used previously (12, 20, 25). It was relatively pure, showing an intense band of 29 kDa and two very faint bands of 10 and 17 kDa on sodium dodecyl sulfate-polyacrylamide gel electrophoresis. BALB/cJ, C3H/HeJ, and C3H/HeN mice were obtained from the Jackson Laboratories, Bar Harbor, Maine. They were primed with ACT-D mannitol (Sigma Chemical Co., St. Louis, Mo.) by intraperitoneal injection; this was followed by intravenous or intraperitoneal SEB challenge. Both ACT-D and SEB were dissolved in pyrogen-free normal saline, and 0.4 ml of the solutions was injected into each mouse. Mice were observed for signs of illness and sacrificed at the terminal stage or at an appropriate time point; their tissues were removed and processed for histopathological studies.

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TABLE 1. Increased sensitivity to SEB-induced lethal shock in BALB/cJ mice primed with ACT-D^a

ACT-D (μ g/mouse)	SEB (μ g/mouse)	No. of mice that died/total tested	P value ^b	Remarks
0	100	0/4		Sick
0	500	1/5	0.556	Died in 1 day
0	1,000	5/5	0.00794	Died in 1 day
10	0	0/4		No signs of illness
10	10	0/4		Sick
10	20	0/4		Sick
10	50	0/5		Sick
10	100	0/4		Sick
20	0	0/4		No signs of illness
20	10	0/4		Sick
20	20	1/5	0.556	Died in 4 days
20	50	4/5	0.0397	Died in 2-4 days
20	100	4/5	0.0397	Died in 2-3 days

^a Mice were primed with ACT-D intraperitoneally. Twenty minutes later, the mice were challenged intraperitoneally with SEB and were observed for 1 week for signs of illness and survival and death.

^b P values were calculated by Fisher's exact test for 2 \times 2 tables. Comparisons were between mice given ACT-D plus SEB and mice given the same amount of ACT-D but no SEB.

Monkey tissues and histological sections were from previous studies in which Wallace Baze was the pathologist.

BALB/cJ mice are commonly used in immunological studies. Their T and B cells as well as macrophages have been extensively described. If activation of antigen-presenting cells, T cells, and other lymphoid cells is the main cause of SEB toxicosis, toxic shock, and death (10, 13, 14), BALB/cJ mice would provide an advantage over other animal species for studies of pathogenesis. Thus, to develop a mouse model, BALB/cJ mice were tested first. They were intraperitoneally primed with ACT-D, intravenously or intraperitoneally challenged with various doses of SEB, and observed for illness and ultimate outcome. Intraperitoneal and intravenous challenges with SEB showed essentially the same results. A representative result is summarized in Table 1. BALB/cJ mice were highly resistant to lethal toxic shock when given SEB alone. A dose of more than 500 μ g per mouse was generally required. When BALB/cJ mice were primed with 10 μ g of ACT-D per mouse and challenged with 100 μ g of SEB, they became sick (see below), but they recovered. However, when BALB/cJ mice were primed with 20 μ g of ACT-D per mouse, lethality began to be seen at a SEB dose of 20 μ g per mouse (one of five mice died). When the doses of SEB were increased to 50 and 100 μ g per mouse in these ACT-D-primed mice, 80% (four of five mice) died. These results suggest that BALB/cJ mice primed with ACT-D became susceptible to lethal SEB-induced toxic shock.

Although no deaths occurred when mice were challenged only with low doses of SEB or were primed with a low dose (10 μ g per mouse) of ACT-D and then challenged with a low dose of SEB, the mice generally appeared sick in the first 24 h (Table 1). They were inactive, stopped drinking and eating, and gathered close to one another. In severe cases, the mice had ruffled fur and shook while walking, with a splinting abdomen. Mice primed only with ACT-D or with saline appeared healthy and did not show such signs of illness. On the other hand, the dying mice that had been intoxicated with a high dose of SEB or that had been primed with ACT-D and challenged with a lower dose of SEB generally showed four stages of toxicosis. Shortly (1 to 2 h) after SEB challenge, they

TABLE 2. Increased sensitivity of C3H/HeJ mice to SEB lethality by priming with ACT-D^a

ACT-D (μ g/mouse)	SEB (μ g/mouse)	No. of mice that died/total tested	P value ^b	Remarks
0	100	0/5		Sick
10	0	0/5		No signs of illness
10	50	0/5		Sick
10	100	4/5	0.024	Died in 2-3 days
20	0	0/5		No signs of illness
20	50	4/8	0.098	Died in 2-6 days
20	100	5/5	0.00397	Died in 1 day
30	0	0/5		Sick at days 3-4
30	50	5/5	0.00397	Died in 1 day
30	100	5/5	0.00397	Died in 1 day

^a C3H/HeJ mice were primed with ACT-D 20 min before SEB challenge. ACT-D-primed mice challenged with normal saline (no SEB) served as controls. ACT-D, SEB, or saline was administered intraperitoneally.

^b P values were calculated by Fisher's exact test for 2 \times 2 tables. Comparisons were between mice given ACT-D plus SEB and mice given the same amount of ACT-D but no SEB.

became inactive and gathered close to each other. Later, they trembled, with a splinting abdomen; isolated themselves; and displayed increased lethargy. Subsequently, at 24 to 72 h after SEB challenge, the mice went into coma. They collapsed on the floor, showed rapid and short breathing, and did not respond at all to the touch of a pencil, indicating a stage of toxic shock. A fatal outcome followed shortly (30 to 120 min) after the onset of this comatose stage. Mice that did not undergo the comatose stage began to recover 3 to 4 days later.

In rabbits intoxicated with SEB, significant levels of bacterial LPS have been detected in the blood circulation (17). It has been suggested that the LPS from endogenous, gram-negative bacteria in the gut may have a synergistic effect with SEB, causing intoxication (17, 24). To minimize or eliminate the influence of LPS, an experiment similar to the one described above with BALB/cJ mice was conducted in C3H/HeJ mice, which are resistant to LPS activation and LPS-induced toxic shock (19, 22, 27). The results are summarized in Table 2. C3H/HeJ mice were somewhat more sensitive than BALB/cJ mice to SEB after priming with ACT-D. When primed with 10 μ g of ACT-D per mouse, C3H/HeJ mice were sensitive to SEB at 100 μ g per mouse, while BALB/cJ mice at the same ACT-D and SEB doses were not sensitive (Tables 1 and 2). Although priming with a higher dose of ACT-D (30 μ g per mouse) further increased the lethality of SEB in C3H/HeJ mice (Table 2), death was quicker. However, what appeared to be increased sensitivity to SEB may in fact have been the direct toxic effect of ACT-D or an additive effect of the toxicity of SEB and the toxicity of a high dose of ACT-D.

In contrast to C3H/HeJ mice, C3H/HeN mice are sensitive to LPS activation and toxic shock (19, 22, 27); an additive effect of SEB and LPS should have been seen in the C3H/HeN mice after SEB intoxication if LPS is essential in SEB-induced toxic shock. To further study the possibility that the lethal toxicosis of C3H/HeJ mice is mainly due to the effect of SEB rather than to the additive effect of SEB and endogenous LPS, a comparative study of C3H/HeJ and C3H/HeN mice was conducted. The results showed that C3H/HeJ and C3H/HeN mice had essentially the same sensitivity to SEB whether or not they were primed with ACT-D (data not shown). Thus, LPS from the gut may not be an essential cofactor in SEB-induced lethal intoxication in mice.

TABLE 3. Time course of histopathological changes in the lung, intestine, spleen, and liver of ACT-D-primed C3H/HeJ mice intoxicated with SEB

Time (h) ^a	Histopathological changes in:			
	Lung	Intestine	Spleen	Liver
4	Blood congestion; PMN pavingmenting	Blood congestion; PMN pavingmenting; mild edema in the lamina propria	Mild blood congestion; large numbers of PMNs in the red pulp	Mild blood congestion; PMNs in sinusoid
8	Blood congestion; PMN pavingmenting; thickening of alveolar septa	Same as above	Great numbers of PMNs in the red pulp; mitotic lymphocytes easily seen	Blood congestion; focal necrosis of hepatocytes; mild to moderate inflammation with PMNs
16	Blood congestion; PMN pavingmenting and widening of alveolar septa, with increased cellularity	Blood congestion; epithelial flattening with villous blunting; lymphocyte necrosis in Peyer's patches	Prominent lymphocyte necrosis and macrophage phagocytosis; PMN increase in the red pulp	Same as above
36-72	Same as above	Same as above	Same as above, plus lymphocyte depletion	Same as above

^a C3H/HeJ mice were primed with ACT-D and challenged with SEB intraperitoneally. At 4, 8, and 16 h, a group of four to five mice was sacrificed. Mice that went into shock in 36 to 72 h were sacrificed and grouped together. Tissues were processed for histopathological studies.

Pathological information derived from monkeys and humans in SEB toxic shock cases is mainly from postmortem studies. There is no information concerning the time course of pathological changes under SEB toxicosis. Our ACT-D-primed mice allowed us to conduct a time course study. For this purpose, C3H/HeJ mice were intraperitoneally primed with ACT-D (10 µg per mouse) and then intraperitoneally challenged with SEB (100 µg per mouse). At timed intervals, mice were observed for signs of illness, and groups of four to five mice were sacrificed for pathologic studies. Mice that went into shock (the comatose stage) at different times (36 to 72 h) were sacrificed and grouped together as one time point. Mice primed with ACT-D and injected with normal saline served as controls. Anatomical and microscopic pathological features of the C3H/HeJ mice are summarized as follows. Anatomically, the controls generally showed no visible pathological changes. However, mice primed with ACT-D and challenged with SEB showed several prominent gross pathologic reactions, as follows. (i) The duodenum and upper jejunum segmentally dilated as early as 4 h after SEB challenge. This dilated part of the gut was filled with light yellowish liquid and undigested food. The dilated portion of the gut became longer, forming the shape of a long balloon, and extended down to the lower jejunum as time passed. The ileum appeared to be constricted. At later times (36 to 72 h), the ballooned gut segments became somewhat more yellowish. Peyer's patches were congested with blood, which turned a dark brown color. (ii) A substantial amount (0.5 to 0.8 ml) of pleural exudate (light yellowish) was seen at 16 h after SEB challenge; however, the exudate was reduced (0.1 to 0.2 ml) at later times (36 to 72 h). (iii) Blood congestion in the lung and intestine was consistently seen during toxicosis, and the blood turned brown and then dark brown at later times (36 to 72 h). (iv) The size of the spleen and the thymus was slightly reduced at 16 h after SEB challenge, and marked atrophy was seen at 36 to 72 hours compared with controls.

In mice primed with ACT-D and challenged with SEB, prominent histopathological changes were seen in the lung, small intestine, and lymphoid tissues such as the spleen. A brief time course description of pathological changes in the lung, intestine, spleen, and liver is given in Table 3, and prominent

pathological features in the lung, small intestine, and spleen are shown in Fig. 1. In the lung, congestion in the venules and capillaries and "pavingment" with polymorphonuclear leukocytes (PMNs) to the vessel wall were noticeable as early as 4 h after SEB challenge. This reaction was followed (at 8 h) by the thickening of the alveolar septa due to increased cellularity (Fig. 1A), which became more severe later (16 to 72 h). In the small intestine, the prominent features were blood congestion and PMN pavingmenting and infiltration, which were consistently seen at all times after SEB challenge. These features were later (at 16 h) followed by blood congestion, epithelial flattening with villous blunting (Fig. 1B), and lymphocyte necrosis in the Peyer's patches, which persisted to the comatose stage (36 to 72 h). The first reactions in the spleen were mild blood congestion and the appearance of many PMNs in the red pulp at 4 h after challenge. At 8 h after challenge, the PMNs increased in numbers, and mitotic lymphocytes appeared in both the red pulp and the white pulp. Subsequently (at 16 h), many necrotic lymphocytes and macrophages with phagocytosed materials appeared in both the red pulp and the white pulp (Fig. 1C). At the comatose stage (36 to 72 h), lymphocytes in the white pulp were depleted, resulting in an appearance of increased reticular cells. The reactions in the liver were mainly slight blood congestion and mild inflammatory reactions with predominant PMNs, followed occasionally by a focal necrosis of hepatocytes. In addition to these prominent pathological changes, parenchymal cell degeneration could also be seen in the lung, intestine, liver, and spleen.

The histopathological reactions in the ACT-D-primed and SEB-challenged mice were compared with those of rhesus monkeys challenged with intravenous SEB. A comparison of the histopathologic findings is summarized in Table 4. Although there were differences, similarities were many. In both the mice and the monkeys, similar pictures of blood congestion and hemorrhage, PMN pavingmenting, and inflammatory cell infiltration were prominent in the lung and intestine. Also, in the lung, thickening of the alveolar septa due to increased cellularity was seen in both the monkeys and the mice. Intraalveolar edema was only seen in the monkeys, whereas hydrothorax was seen in mice. Both the mice and the monkeys

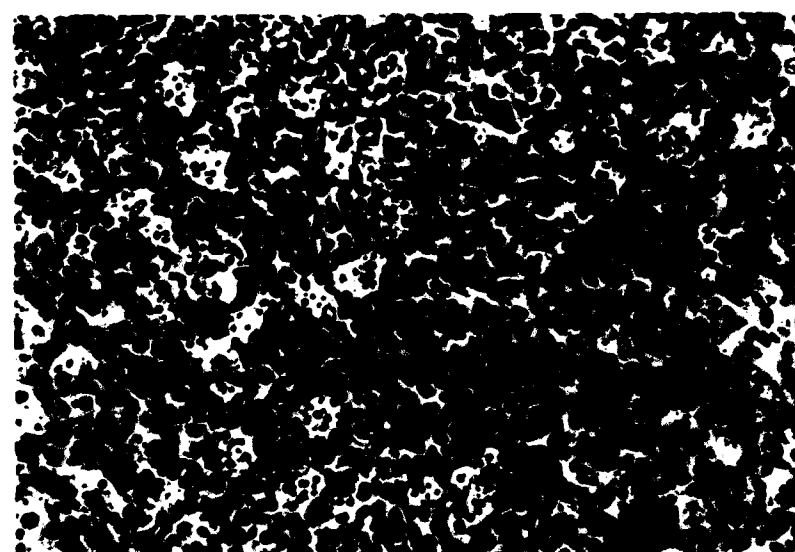
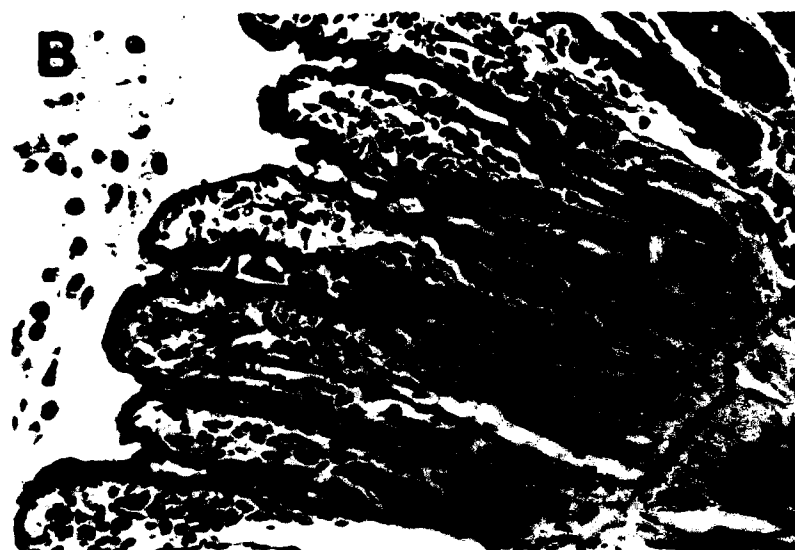
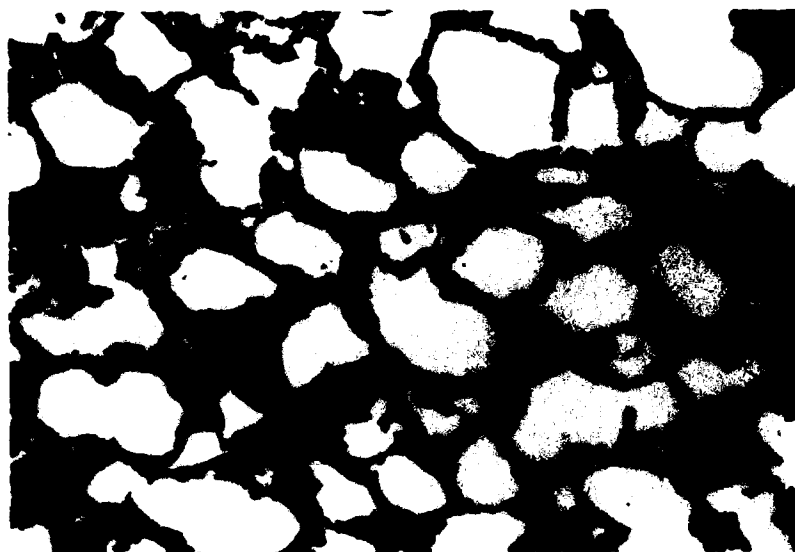


FIG. 1. Pathological reactions in the lung, small intestine, and spleen of C3H/HeJ mice primed with ACT-D (10 μ g per mouse) and challenged with SEB (100 μ g per mouse). (A) Thickening of the alveolar septa (8 h after SEB challenge; hematoxylin and eosin stain; magnification, $\times 50$). (B) Epithelium flattening with villous blunting of the ileum (24 h after SEB challenge; hematoxylin and eosin stain; magnification, $\times 100$). (C) Necrotic lymphocytes and macrophages with phagocytosed materials in the spleen (16 h after SEB challenge; hematoxylin and eosin stain; magnification, $\times 100$).

TABLE 4. Comparison of major histopathological changes in monkeys and ACT-D-primed C3H/HeJ mice intoxicated with SEB

Change no.	Histopathology ^a	Occurrence ^b in:	
		Monkeys ^c	ACT-D-primed mice ^d
1	Congestion and hemorrhage	+	+
2	Leukocyte paving	+	+
3	Inflammatory cell infiltration	+	+
4	Exudation (lung):		
	Intra-alveolar edema	+	-
	Alveolar septum thickening	+	+
	Hydrothorax	-	+
5	Parenchymal cell degeneration	+	+
6	Lymphocyte necrosis	+	++
7	Increase of mitotic lymphocytes	+	+
8	Macrophage phagocytosis	+	++
9	Hepatocyte necrosis (focal)	+/-	+
10	Epithelium flattening with villous blunting (gut)	+	+

^a Histopathological changes 1 to 3 were mostly found in the lung and small intestine; pathological change 5 was found mainly in the lung, intestine, spleen, and liver; and pathological changes 6 to 8 occurred in lymphoid tissues such as the spleen and Peyer's patches.

^b +, easily seen; ++, very prominent; -, not seen.

^c Monkeys were intravenously challenged with 1 mg of SEB in saline per kg of body weight.

^d C3H/HeJ mice were primed with ACT-D (10 µg per mouse) and challenged with SEB (100 µg per mouse). Both ACT-D and SEB were administered intraperitoneally.

had parenchymal cell degeneration in various tissues. Mice generally had more prominent macrophage phagocytosis and lymphocyte necrosis. Although focal hepatocyte necrosis could be easily found in mice, it was only occasionally seen in monkeys. Ileal epithelial flattening with villous blunting was prominent in both the monkeys and the mice.

In summary, we have shown in the present study that mice (BALB/cJ, C3H/HeJ, and C3H/HeN) primed with ACT-D become very susceptible to SEB-induced lethal intoxication. The mice underwent toxicosis and toxic shock and died. This increased sensitivity to SEB is not due to the influence of LPS derived from the gram-negative bacteria in the gut. Prominent pathological changes were seen in the lungs, intestine, and lymphoid tissues such as the spleen. These pathological changes in the mice had many similarities with those in rhesus monkeys challenged with SEB intravenously. The ACT-D-primed mice, particularly the LPS-resistant C3H/HeJ mice, are thus a good small animal model for studying the pathogenesis mechanism of SEB toxicosis and toxic shock. The mouse model provides an advantage in that it allows one to perform studies of the time course of pathological changes, which are difficult to perform in monkeys and impossible to perform in humans.

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